

**Supplementary Table 1.** Mutator phenotype caused by *msh3* alleles as observed by patch test in MMR assays. wt, indicates a rate similar to wild-type, null, indicates a rate similar to the vector alone. Partial indicates an intermediate phenotype.

**Supplementary Table 2.** Mutation rate caused by *msh3* alleles in the *hom3-10* frameshift reversion and 4 nucleotide microsatellite stability assays. Rate is shown quantitatively if measured, or qualitatively if observed by patch test. The increase relative to the value of the wild-type is shown. Ninety-five percent confidence intervals are shown in brackets. Fourteen independent isolates were used to calculate each value.

**Supplementary Table 3.** Oligonucleotides used to create *msh3* and *msh6* point mutations. Sequences are shown in the 5' to 3' direction. The codon substitution is shown in lower case letters.

**Supplementary Table 4.** Stereochemical analysis of the of the Msh3 mispair-binding domain homology model. The parameters listed provide a measure of the accuracy of the molecular models illustrated in Figures 1 and 4.

**Supplementary Table 5.** *S. cerevisiae* strains and plasmids used in this study. Strain numbers and the corresponding relevant genotypes are shown, as well as the original source of the strains or plasmids.

**Supplementary Figure 1.** Alignment of the MutS homologue protein sequences from various organisms. Numbers following each line of the alignment are the amino acid positions of the last amino acids displayed on each line. "+" above the position indicates a position where mutations were made and tested in this study. Asterisks below the position indicate an amino acid residue conserved in Msh3, Msh6 and MutS proteins.

**Supplementary Figure 2.** Suppression of the *msh3Δ* phenotype by alternate amino acid substitutions in *msh3* mutant alleles in MMR assays. (a) Patches of *msh3Δ msh6Δ* strains expressing the indicated *msh3* alleles were replica plated onto -threonine plates for the -1 nucleotide *hom3-10* reversion assay. Patches of *msh3Δ msh6Δ* strains expressing the indicated *msh3* alleles and containing a microsatellite plasmid with an in frame 4 nucleotide repeat sequence upstream of the *URA3* gene were replica plated onto -leucine – tryptophan +uracil +5-fluoroorotic acid plates as shown.

**Supplementary Figure 3.** Phenotype caused by *msh6* mutant alleles in the *hom3-10* reversion assay. The indicated *msh6* alleles were expressed on a low copy-number plasmid bearing the original promoter sequence and a marker allowing growth on -leucine media. Plasmids were transformed into the *msh3Δ msh6Δ* strain and isolates were patched onto -leucine plates, then replica plated onto -threonine plates as shown for the -1 nucleotide *hom3-10* reversion assay.

**Supplementary Figure 4.** Phenotype caused by *msh3* mutant alleles in MMR assays. The indicated *msh3* alleles were expressed on a low copy-number plasmid bearing the original promoter sequence and a marker allowing growth on –leucine media. Plasmids were transformed into the *msh3Δ msh6Δ* strain and isolates were patched onto –leucine plates, then replica plated onto –threonine plates as shown for the -1 nucleotide *hom3-10* reversion assay. For the microsatellite stability assay, a plasmid containing a 4 nucleotide repeat sequence inserted in frame and prior to the *URA3* gene was transformed into the *msh3Δ msh6Δ* strain containing a *msh3* allele on a low-copy number *LEU2* plasmid and patched onto –leucine -tryptophan, then replica plated onto –leucine –tryptophan +uracil +5-fluoroorotic acid plates as shown.

**Supplementary Figure 5.** Mutant Msh3 proteins are expressed at wild-type levels. An *msh3Δ msh6Δ* strain containing plasmids with the indicated 6xHA tagged *MSH3* genes was analyzed for expression of the indicated Msh3 proteins by immunoprecipitation with anti-HA antibody followed by Western blot analysis with anti-HA antibody. The upper panel shows the analysis of HA-tagged Msh3 and the lower panel shows the analysis of the starting extracts with anti-Cdc11 antibody to show that the starting extracts contained equivalent amounts of protein. (a) analysis of the vector control and Msh3, Msh3-Y157S, Msh3-K158D and Msh3-K160D proteins. (b) analysis of the vector control and Msh3, Msh3-Y199A, Msh3-S201G and Msh3-R206A proteins; the black vertical line indicates

the place where the lane containing a duplicate Msh3-K160D sample was excised from the image. The positions where different molecular weight markers migrated on each gel are indicated on the left.

Supplementary Table 1. Phenotypic characterization of frameshift reversion and microsatellite instability in *msh3* strains

Genotype	frameshift	2 nt loop	4 nt loop
MSH3	wt	wt	wt
vector	null	null	null
<i>msh3-Y157S</i>	partial	wt	wt
<i>msh3-K158D</i>	null	null	null
<i>msh3-K160D</i>	null	null	null
<i>msh3-F162A</i>	partial	partial	partial
<i>msh3-E164A</i>	wt	wt	wt
<i>msh3-R171A</i>	wt	wt	wt
<i>msh3-H174A</i>	wt	wt	wt
<i>msh3-H194E</i>	wt	wt	wt
<i>msh3-R195D</i>	null	wt	wt
<i>msh3-F197A</i>	partial	partial	partial
<i>msh3-Y199A</i>	partial	wt	wt
<i>msh3-S201G</i>	partial	wt	wt
<i>msh3-R206A</i>	partial	wt	wt
<i>msh3-H210A</i>	null	null	partial
<i>msh3-ERN</i>	null	null	null

Supplementary Table 2. Rates of frameshift reversion and microsatellite instability in *msh3* strains

Genotype	Frameshift		4 bp unit	
	Mutation Rate	relative rate	Mutaton Rate	relative rate
MSH3	2.1 [0.9-4.5] × 10 <sup>-8</sup>	1	9.5 [4.5-24.8] × 10 <sup>-6</sup>	1
vector	9.6 [5.2-13.3] × 10 <sup>-6</sup>	457	2.5 [0.8-8.5] × 10 <sup>-4</sup>	26
<i>msh3-Y157S</i>	2.7 [1.1-3.5] × 10 <sup>-6</sup>	129	1.3 [0.9-3.7] × 10 <sup>-5</sup>	1.4
<i>msh3-Y157F</i>	3.5 [1.5-7.0] × 10 <sup>-6</sup>	166	1.8 [1.2-4.0] × 10 <sup>-5</sup>	1.9
<i>msh3-Y157L</i>	7.1 [5.3-10.0] × 10 <sup>-6</sup>	338	1.1 [0.7-2.1] × 10 <sup>-5</sup>	1.2
<i>msh3-Y157D</i>	2.3 [0.9-3.5] × 10 <sup>-5</sup>	1095	1.2 [0.6-7.0] × 10 <sup>-4</sup>	13
<i>msh3-Y157A</i>	2.1 [1.4-3.7] × 10 <sup>-6</sup>	100	1.8 [0.4-4.3] × 10 <sup>-5</sup>	1.9
<i>msh3-K158D</i>	1.6 [1.1-2.5] × 10 <sup>-5</sup>	761	2.5 [1.8-6.0] × 10 <sup>-4</sup>	26
<i>msh3-K158A</i>	1.4 [0.4-2.8] × 10 <sup>-6</sup>	66	2.0 [1.5-4.6] × 10 <sup>-5</sup>	2.1
<i>msh3-K158R</i>	3.2 [1.2-7.3] × 10 <sup>-8</sup>	1.5	2.1 [0.7-3.3] × 10 <sup>-5</sup>	2.2
<i>msh3-K158E</i>	2.2 [1.4-3.6] × 10 <sup>-5</sup>	1047	8.4 [2.5-18.1] × 10 <sup>-4</sup>	88
<i>msh3-K158M</i>	8.3 [5.1-13.1] × 10 <sup>-7</sup>	39	1.7 [1.1-2.9] × 10 <sup>-5</sup>	1.8
<i>msh3-Y157A K158A</i>	1.9 [0.9-2.9] × 10 <sup>-6</sup>	90	4.3 [2.3-13.0] × 10 <sup>-6</sup>	0.5
<i>msh3-K160D</i>	null		null	
<i>msh3-F162A</i>	5.4 [3.2-20.0] × 10 <sup>-6</sup>	257	5.9 [3.7-9.5] × 10 <sup>-5</sup>	6.2
<i>msh3-F162Y</i>	3.8 [2.5-10.1] × 10 <sup>-7</sup>	18	1.9 [0.9-4.7] × 10 <sup>-5</sup>	2
<i>msh3-F162S</i>	7.6 [4.0-15.7] × 10 <sup>-6</sup>	362	2.0 [0.9-5.2] × 10 <sup>-4</sup>	21
<i>msh3-E164A</i>	wt		wt	
<i>msh3-R171A</i>	wt		wt	
<i>msh3-H174A</i>	wt		wt	
<i>msh3-H194E</i>	wt		wt	
<i>msh3-R195D</i>	4.1 [1.7-7.9] × 10 <sup>-6</sup>	195	2.4 [0.9-5.9] × 10 <sup>-5</sup>	2.5
<i>msh3-F197A</i>	5.0 [3.6-8.4] × 10 <sup>-6</sup>	238	1.8 [0.6-3.2] × 10 <sup>-4</sup>	19
<i>msh3-F197H</i>	2.4 [1.5-6.6] × 10 <sup>-6</sup>	114	4.5 [3.2-5.3] × 10 <sup>-5</sup>	4.7
<i>msh3-Y199A</i>	2.5 [2.1-6.3] × 10 <sup>-6</sup>	119	3.9 [1.0-8.7] × 10 <sup>-5</sup>	4.1
<i>msh3-Y199L</i>	null		partial	
<i>msh3-Y199D</i>	null		null	
<i>msh3-Y199K</i>	partial		partial	
<i>msh3-S201G</i>	8.2 [5.6-11.4] × 10 <sup>-7</sup>	39	1.9 [0.8-3.4] × 10 <sup>-5</sup>	2
<i>msh3-S201L</i>	partial		partial	
<i>msh3-S201D</i>	null		null	
<i>msh3-S201R</i>	null		null	
<i>msh3-R206A</i>	5.2 [1.7-11.3] × 10 <sup>-6</sup>	247	2.0 [1.3-3.3] × 10 <sup>-5</sup>	2.1
<i>msh3-H210A</i>	null		partial	
<i>msh3-ERN</i>	null		null	
Msh6	wt		null	
vector	null		null	
<i>msh6-G368S</i>	null		null	
<i>msh6-S373R</i>	wt		null	

Supplementary Table 3. Stereochemical analysis of the Msh3 mispair-binding domain homology model.

Parameter	Entire Model (residues 133-255)	High Confidence Regions of Model (residues 133-174, 194-229, 245-255)
<i>Ramachandran Plot*</i>		
Most Favored Regions	83.2%	90.0%
Additional Allowed Regions	13.3%	7.5%
Generously Allowed Regions	1.8%	1.3%
Disallowed Regions	1.8%	1.3%
<i>Comparison to 2.0 Å structures*</i>		
Ramachandran parameters	Inside (-0.1 bwfm <sup>†</sup> )	Inside (0.6 bwfm <sup>†</sup> )
Omega angle std dev	Better (-1.7 bwfm <sup>†</sup> )	Better (-1.7 bwfm <sup>†</sup> )
Bad contacts/100 residues	Inside (-0.4 bwfm <sup>†</sup> )	Inside (-0.4 bwfm <sup>†</sup> )
Zeta angle std dev	Better (-1.2 bwfm <sup>†</sup> )	Better (-1.3 bwfm <sup>†</sup> )
H-bond energy std dev	Inside (-0.3 bwfm <sup>†</sup> )	Inside (-0.3 bwfm <sup>†</sup> )
Overall G-factor	Better (+2.1 bwfm <sup>†</sup> )	Better (+2.3 bwfm <sup>†</sup> )
<i>Root Mean Squared Deviation<sup>‡</sup></i>		
Bonds (Å)	0.004	0.004
Angles (°)	1.6	1.6

\*As reported by Procheck. Note the Ramachandran outlier, Ala163, has the same backbone conformation as the Ramachandran outlier His437 in the human Msh6 structure (2o8b).

<sup>†</sup> BandWidths From Mean.

<sup>‡</sup>As reported by CNS\_SOLVE.

Supplementary Table 4. Oligonucleotides used to create *msh3* and *msh6* point mutations.

Msh3		
codon	Oligo	
Position	Name	Sequence
Y157S	JH145	AGAGATAAAGTGCTTGTATTAGAGTAGGC <sup>agc</sup> AAGTACAAATGTTGCAGAGGATGCAGT
	JH146	ACTGCATCCTCTGCAAAACATTGTACTT <sup>gc</sup> GCCTACTCTAATAACAAGC <sup>ACT</sup> TATCTCT
Y157F	JH165	AGAGATAAAGTGCTTGTATTAGAGTAGGC <sup>tt</sup> AAGTACAAATGTTGCAGAGGATGCAGT
	JH166	ACTGCATCCTCTGCAAAACATTGTACTT <sup>a</sup> GCCTACTCTAATAACAAGC <sup>ACT</sup> TATCTCT
Y157L	JH167	AGAGATAAAGTGCTTGTATTAGAGTAGGC <sup>c</sup> tAAGTACAAATGTTGCAGAGGATGCAGT
	JH168	ACTGCATCCTCTGCAAAACATTGTACTT <sup>a</sup> gGCCTACTCTAATAACAAGC <sup>ACT</sup> TATCTCT
Y157D	JH169	AGAGATAAAGTGCTTGTATTAGAGTAGGC <sup>g</sup> atAAGTACAAATGTTGCAGAGGATGCAGT
	JH170	ACTGCATCCTCTGCAAAACATTGTACTT <sup>a</sup> cGCCTACTCTAATAACAAGC <sup>ACT</sup> TATCTCT
Y157A	JH171	AGAGATAAAGTGCTTGTATTAGAGTAGGC <sup>g</sup> caAAGTACAAATGTTGCAGAGGATGCAGT
	JH172	ACTGCATCCTCTGCAAAACATTGTACTT <sup>t</sup> <sup>g</sup> GCCTACTCTAATAACAAGC <sup>ACT</sup> TATCTCT
K158D	JH147	GATAAAGTGCTTGTATTAGAGTAGGC <sup>T</sup> AC <sup>g</sup> atTACAAATGTTGCAGAGGATGCAGTAAC
	JH148	GT <sup>T</sup> ACTGCATCCTCTGCAAAACATTGT <sup>A</sup> tcGTAGC <sup>C</sup> TACTCTAATAACAAGC <sup>ACT</sup> TATC
K158A	JH173	GATAAAGTGCTTGTATTAGAGTAGGC <sup>T</sup> AC <sup>g</sup> caTACAAATGTTGCAGAGGATGCAGTAAC
	JH174	GT <sup>T</sup> ACTGCATCCTCTGCAAAACATTGT <sup>A</sup> t <sup>g</sup> GTAGC <sup>C</sup> TACTCTAATAACAAGC <sup>ACT</sup> TATC
K158R	JH175	GATAAAGTGCTTGTATTAGAGTAGGC <sup>T</sup> AC <sup>g</sup> aTACAAATGTTGCAGAGGATGCAGTAAC
	JH176	GT <sup>T</sup> ACTGCATCCTCTGCAAAACATTGT <sup>A</sup> t <sup>c</sup> GTAGC <sup>C</sup> TACTCTAATAACAAGC <sup>ACT</sup> TATC
K158E	JH177	GATAAAGTGCTTGTATTAGAGTAGGC <sup>T</sup> AC <sup>g</sup> gTACAAATGTTGCAGAGGATGCAGTAAC

	JH178	GTTACTGCATCCTCTGCAAAACATTGTActcGTAGCCTACTCTAATAACAAGCACTTTATC
K158M	JH179	GATAAAAGTGCTTGTATTAGAGTAGGCTACatgTACAAATGTTGCAGAGGATGCAGTAAC
	JH180	GTTACTGCATCCTCTGCAAAACATTGTAcgtGTAGCCTACTCTAATAACAAGCACTTTATC
Y157A K158A	JH200	AGAGATAAAAGTGCTTGTATTAGAGTAGGCgcagcaTACAAATGTTGCAGAGGATGCAGTAAC
	JH201	GTTACTGCATCCTCTGCAAAACATTGTAtgctgcGCCTACTCTAATAACAAGCACTTTATCTCT
K150D	JH149	GTGCTTGTATTAGAGTAGGCTACAAGTACAGTACgtTGTTGCAGAGGATGCAGTAACGGTTAGC
	JH150	GCTAACCGTTACTGCATCCTCTGCAAAACAAatcGTACTTGTAGCCTACTCTAATAACAAGCAC
F162A	JH129	GTTATTAGAGTAGGCTACAAGTACAAATGTgcaGCAGAGGATGCAGTAACGGTTAGCAGAATA
	JH130	TATTCTGCTAACCGTTACTGCATCCTCTGCTgcACATTGTACTTGTAGCCTACTCTAATAAC
F162Y	JH181	GTTATTAGAGTAGGCTACAAGTACAAATGTtacGCAGAGGATGCAGTAACGGTTAGCAGAATA
	JH182	TATTCTGCTAACCGTTACTGCATCCTCTGCgtACATTGTACTTGTAGCCTACTCTAATAAC
F162S	JH183	GTTATTAGAGTAGGCTACAAGTACAAATGTtagcGCAGAGGATGCAGTAACGGTTAGCAGAATA
	JH184	TATTCTGCTAACCGTTACTGCATCCTCTGCgtACATTGTACTTGTAGCCTACTCTAATAAC
E164A	JH131	AGAGTAGGCTACAAGTACAAATGTTGCAGcaGATGCAGTAACGGTTAGCAGAATACTTCAC
	JH132	GTGAAGTATTCTGCTAACCGTTACTGCATCTgctGCACAAACATTGTACTTGTAGCCTACTCT
R171A	JH133	TGTTTGCAAGAGGATGCAGTAACGGTTAGCgcaATACTCACATCAAACATTGTGCCTGGAAAAA
	JH134	TTTCCAGGCACAAGTTGATGTGAAGTATTgcGCTAACCGTTACTGCATCCTCTGCAAAACA
H174A	JH141	GAGGATGCAGTAACGGTTAGCAGAATACTTgcaATCAAACATTGTGCCTGGAAAATTGACTATC
	JH142	GATAGTCAATTTCAGGCACAAGTTGATTgcAAGTATTCTGCTAACCGTTACTGCATCCTC

H194E	JH155	ATCGATGAGTCTAACCTCAAGATTGCAATgagAGGCAGTTGCGTACTGTTCTTCCGGAT
	JH156	ATCCGGAAAGAACAGTACGCAAAC TGCGCTctcATTGCAATCTTGAGGATTAGACTCATCGAT
R195D	JH157	GATGAGTCTAACCTCAAGATTGCAATCATgatCAGTTGCGTACTGTTCTTCCGGATGTC
	JH158	GACATCCGGAAAGAACAGTACGCAAAC TGatcATTGCAATCTTGAGGATTAGACTCATC
F197A	JH137	TCTAATCCTCAAGATTGCAATCATAGGCAGGcaGCGTACTGTTCTTCCGGATGTCAGATTA
	JH138	TAATCTGACATCCGGAAAGAACAGTACGCTgcCTGCCTATGATTGCAATCTTGAGGATTAGA
F197H	JH185	TCTAATCCTCAAGATTGCAATCATAGGCAGcatGCGTACTGTTCTTCCGGATGTCAGATTA
	JH186	TAATCTGACATCCGGAAAGAACAGTACGCatgCTGCCTATGATTGCAATCTTGAGGATTAGA
Y199A	JH143	CCTCAAGATTGCAATCATAGGCAGTTGCGGcaTGTCTTCCGGATGTCAGATTAAACGTT
	JH144	AACGTTTAATCTGACATCCGGAAAGAACAtgcCGCAAAC TGCGCTATGATTGCAATCTTGAGG
Y199L	JH219	CCTCAAGATTGCAATCATAGGCAGTTGCGttaTGTCTTCCGGATGTCAGATTAAACGTT
	JH220	AACGTTTAATCTGACATCCGGAAAGAACAtaaCGCAAAC TGCGCTATGATTGCAATCTTGAGG
Y199D	JH221	CCTCAAGATTGCAATCATAGGCAGTTGCGgatTGTCTTCCGGATGTCAGATTAAACGTT
	JH222	AACGTTTAATCTGACATCCGGAAAGAACAtcCGCAAAC TGCGCTATGATTGCAATCTTGAGG
Y199K	JH223	CCTCAAGATTGCAATCATAGGCAGTTGCGaaaTGTCTTCCGGATGTCAGATTAAACGTT
	JH224	AACGTTTAATCTGACATCCGGAAAGAACAtttCGCAAAC TGCGCTATGATTGCAATCTTGAGG
S201G	JH135	GATTGCAATCATAGGCAGTTGCGTACTGTggTTCCCGGATGTCAGATTAAACGTTCACCTA
	JH136	TAGGTGAACGTTAAC TGTGACATCCGGAAaccACAGTACGCAAAC TGCGCTATGATTGCAATC
S201L	JH229	GATTGCAATCATAGGCAGTTGCGTACTGTttaTCCCGGATGTCAGATTAAACGTTCACCTA
	JH230	TAGGTGAACGTTAAC TGTGACATCCGGAAAtaaACAGTACGCAAAC TGCGCTATGATTGCAATC

S201D	JH225	GATTGCAATCATAGGCAGTTGCGTACTGTgatTCGGATGTCAGATTAAACGTTCACCTA
	JH226	TAGGTGAACGTTAACCTGACATCCGGGAAatcACAGTACGCAAACACTGCCTATGATTGCAATC
S201R	JH227	GATTGCAATCATAGGCAGTTGCGTACTGTaggTCGGATGTCAGATTAAACGTTCACCTA
	JH228	TAGGTGAACGTTAACCTGACATCCGGGAAccAcAGTACGCAAACACTGCCTATGATTGCAATC
R206A	JH151	CAGTTGCGTACTGTTCTTCCGGATGTCgcgTTAACGTTCACCTAGAGAGACTTGTGCAT
	JH152	ATGCACAAGTCTCTAGGTGAACGTTAACgcGACATCCGGGAAAGAACAGTACGCAAAC TG
H210A	JH153	TGTTCTTCCGGATGTCAGATTAAACGTTgcgCTAGAGAGACTTGTGCATCATATTAAAG
	JH154	CTTAAATTATGATGCACAAGTCTCTAGgcAACGTTAACCTGACATCCGGGAAAGAAC A
ERN	JH163	AGCAGAATACTCACATCAAACTTGCGCTgagagaaattTGCGTACTGTTCTTCCGGATGTCAGA
	JH164	TCTGACATCCGGAAAGAACAGTACGCAAAttcttcAGGCACAAGTTGATGTGAAGTATTCTGCT
msh6-G368S	JH189	GGTGGAGGACGCGCTAATATGCAACTAGCTcaATTCCAGAGATGTCATTGAATATTGGGCC
	JH190	GGCCAATATTCAAATGACATCTCTGGATTgaAGCTAGTTGCATATTAGCGCGTCCTCCACC
msh6-S373R	JH191	AATATGCAACTAGCTGGATTCCAGAGATGcgTTGAATATTGGCCGCTCAGTTATCAA
	JH192	TTGGATAAACTGAGCGGCCAATATTCAAAGcgCATCTCTGGAAATCCCAGCTAGTTGCATATT

**Supplementary Table 5. *S. cerevisiae* strains and plasmids used in this study.**

Strain No.	Relevant genotype of yeast strain	Other name	Source
RDKY4234	ura3-52, leu2Δ1, trp1Δ63, hom3-10, his3Δ200, lys2-10A, msh3::hisG, msh6::hisG		Ref. 32
RDKY6613	$\Delta ho$ , $\Delta hml1::ADE1$ , $\Delta hmr::ADE1$ , ade1, ade3::Gal::HO, leu2-3, lys5, trp1::hisG, ura3-52	JKM146	Ref. 28
RDKY6859	RDKY6613, msh3::TRP1		This study
RDKY7193	RDKY6613, msh3-Y157S		This study
RDKY7194	RDKY6613, msh3-K160D		This study
RDKY7165	RDKY6613, msh3-Y199A		This study
RDKY7166	RDKY6613, msh3-R206A		This study
Plasmid alleles			
Strain No.		Other name	Source
RDK4055	pRS315 (Amp r, ori, LEU2, ARS-CEN)		Ref. 35
RDK4518	pRS315 MSH3	pRDK1088	Ref. 32
RDK4945	pRS315 msh3-Y157S		This study
RDK5077	pRS315 msh3-Y157F		This study
RDK5071	pRS315 msh3-Y157L		This study
RDK5078	pRS315 msh3-Y157D		This study
RDK5073	pRS315 msh3-Y157A		This study
RDK4935	pRS315 msh3-K158D		This study
RDK5074	pRS315 msh3-K158A		This study
RDK5075	pRS315 msh3-K158R		This study
RDK5076	pRS315 msh3-K158E		This study
RDK5079	pRS315 msh3-K158M		This study
RDK5087	pRS315 msh3-Y157A,K158A		This study
RDK4951	pRS315 msh3-K160D		This study
RDK4925	pRS315 msh3-F162A		This study
RDK5082	pRS315 msh3-F162Y		This study
RDK5081	pRS315 msh3-F162S		This study
RDK4926	pRS315 msh3-E164A		This study
RDK4927	pRS315 msh3-R171A		This study
RDK4952	pRS315 msh3-H174A		This study
RDK4969	pRS315 msh3-H194E		This study
RDK4964	pRS315 msh3-R195D		This study
RDK4928	pRS315 msh3-F197A		This study
RDK5080	pRS315 msh3-F197H		This study
RDK4937	pRS315 msh3-Y199A		This study
RDK5114	pRS315 msh3-Y199L		This study
RDK5115	pRS315 msh3-Y199D		This study
RDK5116	pRS315 msh3-Y199K		This study
RDK4929	pRS315 msh3-S201G		This study
RDK5119	pRS315 msh3-S201L		This study
RDK5117	pRS315 msh3-S201D		This study
RDK5118	pRS315 msh3-S201R		This study
RDK4946	pRS315 msh3-R206A		This study
RDK4954	pRS315 msh3-H210A		This study
RDK4970	pRS315 msh3-ERN		This study
RDK3572	pRS315 MSH6	pRDK439	Ref. 32
RDK5083	pRS315 Msh6-G368S		This study
RDK5085	pRS315 Msh6-S373R		This study

<b>Plasmids for measuring protein expression</b>			
<b>Strain No.</b>		<b>Other name</b>	<b>Source</b>
RDK4055	pRS315 (Amp r, ori, LEU2, ARS-CEN)		Ref. 35
RDK5277	pRS315 MSH3-6HA		This study
RDK5274	pRS315 <i>msh3-F162A-6HA</i>		This study
RDK5300	pRS315 <i>msh3-Y199A-6HA</i>		This study
RDK5275	pRS315 <i>msh3-S201G-6HA</i>		This study
RDK5276	pRS315 <i>msh3-R206A-6HA</i>		This study

<b>Substrate plasmids for 2nt and 4nt microsatellite assay</b>			
<b>Strain No.</b>		<b>Other name</b>	<b>Source</b>
RDK4956	(GT) <sub>16.5</sub>	pSH44	Ref. 34
RDK4957	(CAGT) <sub>16</sub>	pBK1	Ref. 34

<b>Substrate plasmids for DSB repair assay</b>			
<b>Strain No.</b>		<b>Other name</b>	<b>Source</b>
RDK5294	Mismatch (Amp r, ori, URA3, ARS-CEN)	pFP122	Ref. 28
RDK5295	Deletion 30	pFP140	Ref. 28
RDK5120	Deletion 308-610	pFP120	Ref. 28

## **Supplementary Figure 1.**

- 1) MSH3 *Saccharomyces cerevisiae*
- 2) MSH3 *Ustilago maydis*
- 3) MSH3 *Homo sapiens*
- 4) MSH6 *Saccharomyces cerevisiae*
- 5) MSH6 *Homo sapiens*
- 6) MutS *Escherichia coli*
- 7) MutS *Thermus aquaticus*
- 8) MSH3 *Ashbya gossypii*
- 9) MSH3 *Kluyveromyces lactis*
- 10) MSH3 *Candida glabrata*
- 11) MSH3 *Candida albicans*
- 12) MSH3 *Debaryomyces hansenii*
- 13) MSH3 *Yarrowia lipolytica*
- 14) MSH3 *Pongo pygmaeus*
- 15) MSH3 *Pan troglodytes*
- 16) MSH3 *Canus familiaris*
- 17) MSH3 *Mus musculus*
- 18) MSH3 *Gallus gallus*
- 19) MSH3 (SWI4) *Schizosaccharomyces pombe*
- 20) MSH3 *Aspergillus nidulans*
- 21) MSH3 *Aspergillus terreus NIH26*
- 22) MSH3 *Aspergillus oryzae*
- 23) MSH3 *Aspergillus fumigatus*
- 24) MSH3 *Coccidioides immitis*
- 25) MSH3 *Botryotinia fuckeliana*
- 26) MSH3 *Sclerotinia sclerotiorum*
- 27) MSH3 *Chaetomium globosum*
- 28) MSH3 *Podospora anserina DSM 98*
- 29) MSH3 *Neurospora crassa*
- 30) MSH3 *Gibberella zeae*
- 31) MSH3 *Magnaporthe grisea*
- 32) MSH3 *Cryptococcus neoformans B3501A*
- 33) MSH3 *Cryptococcus neoformans JEC21*

1 )	TPLDKQVKDLKMHHRKVLVIRGYKYKCFAEDAVTSRILHIKLVPGKLTIDESN-----PQDCNHRQFAYCSFPDVRL	236
2 )	TPLEQIILELKAEHPGVLLIEVGYKLKFYGEDARIASKELSIMCFPER-----NLNTAMIPVHRL	295
3 )	TPLEQYIEMKQQHKDAVLCVECGYKYRFFGEDAEIAARELNIYCHLDH-----NFMTASIPTHRHL	281
4 )	TPFEKQYWEIKSKMWDCIVFFKKGFELYEKDALLANALFDLKIAGGGRA-----NMQLAGIPEMSF	374
5 )	TPGMRKWWQIKSQNFDLVICYKVGKFYELYHMDALIGVSELGLVFMKG-----NWAHSGFPEIAF	466
6 )	TPMMQQYIQLKAQHPEILLFYRMGDFYELFYDDAKRASQLLDISLTKRGASAGE-----PIPMAGIPYHAV	76
7 )	PPLLQQYVELMKRDQYPDYLLLQVGDFYECFGEDAERLARALGLVLTTSKDF-----TTPMAGIPLRAF	77
8 )	TELDQQFKDLKLQHMDKVLAURVGYKYKFFAEDAVMVSRLQIKLVPGKLTVHETD-----PADHKHKKFAYCTIPDTRL	215
9 )	TELDQQFKELKLKHMDTILCVRVGYKYKFFAKDAEIVSNILQIKLVPGKKTLDSD-----PNDRNYRKFOYCISIPDTRL	224
10 )	TPLDRQVKDLKLGNMDKVLVIRGYKYKIFAQDAAIIASTILHLQLIPGKVTIDDSN-----PNDSKYKQFAYCSFPDVRL	223
11 )	TPLEQIILELTERHQDKILLIQIGYKYKVGFBINALKVSKILNIMYIS-----NDIEDTRFHYSIPDTRL	172
12 )	TPLEQFVEMKQSNLKDILAIQVGYKFKFFGEDAVIASKLSIMLIPGNIKL-----DEYQHDRVAYCSIPDNRL	184
13 )	NATEFQWYEIKKQHRDTLLFVQVGYKYHIYGDAEIAHAQTRLFLSPGTNLKDIGNDGVVQQGSKYIKLAYSSFPVHRI	215
14 )	TPLEQYIEMKQQHKDAVLCVECGYKYRFFGEDAEIAARELNIYCHLDHN-----FMTASIPTHRHL	176
15 )	TPLEQYIEMKQQHKDAVLCVECGYKYRFFGEDAEIAARELNIYCHLDHN-----FMTASIPTHRHL	253
16 )	TPLEQYIELKQQQKDAILCVECGYKYRFFGEDAEIAARELNIYCHLDHN-----FMTASIPTHRHL	279
17 )	TPLEQYLDMKQQHKDAVLCVECGYKYRFFGEDAEIAARELNIYCHLDHN-----FMTASIPTHRHL	248
18 )	TPLELQFIEKKRYKDAILCVECGYKYRFFGEDAEIASKELNICCHQDHN-----FMTASIPTHRHL	242
19 )	TPLEQQYLELKKNYQETILAIEVGYKFRFFGKDAKIASEVLGISCYFEH-----FLNASVPSYRI	164
20 )	TPMEKQVIEIKKKHMDTILLIEVGYKFRFFGEDARIAAKELSIVCIPGKFRYDEHP-----SEAHLDRFASASIPVQRL	269
21 )	TPMERQIIDMKRKHMDSKILAVQVGYKFRFFGEDARVAAKELSIVCIPGKFRFDEHP-----SEAHLDRFASASIPVHKL	280
22 )	TPMEKQVIDIKRKHMDSKILAVQVGYKFRFFGEDARTAAKELNIVCIPGKFRFDEHP-----SEAHLDRFASASIPVHRL	272
23 )	TPLEQVIEIKRKHMDSKILAVQVGYKFRFFGEDARIAAKELSIVCIPGKMRFDEHP-----SEAHLDRFASASIPVHRL	284
24 )	TPLEQVIDEIKRKHMDSKILAVQVGYKFRFFGEDARIAAKELSIVCIPGKLRFDEHP-----SEAHLNRFASASIPVHRL	344
25 )	TPMELQFLEIKRKHMDSKILAVQVGYKFRFFGEDARTAAKVLSSIVCIPGKFRFDEHP-----SESHLNYFASASIPVHRL	284
26 )	TPMELQFLEIKRKHMDSKILAVQVGYKFRFFGEDARTAAKVLSSIVCIPGKFRFDEQ-----AHLNYFASASIPVHRL	281
27 )	TPMEIQFLDIKRKHMDSKILAVQVGYKFRFFGEDARIAAKELSIVCIPGKFRYDEP-----HIDRFASASIPVHRL	298
28 )	TPMEIQFLDIKRKHMDSKILAVQVGYKFRFFGEDARIAAKELSIVCIPGKFRYDEP-----HIDRFASASIPVHRL	282
29 )	TPMELQFLEIKRKHMDSKILAVQVGYKFRFFGEDARIAARELSIVCIPGKFRYDEHP-----SEAHLDRFASASIPVHRL	260
30 )	TPMELQFLEIKRKHMDSKILAVQVGYKFRFFGEDARIAARELSIVCIPGKFRYDEHP-----SEAHLDRFASASIPVHRL	276
31 )	TPMELQFLEIKRKHMDSKILAVQVGYKFRFFGEDARIAAGKELSIVCIPGKFRYDEHP-----SEAHLDRFASASIPVHRL	300
32 )	TPLEQFMEIKEQNRDVLLLMEVGYKYKFHGEGAKTASRELGIVAFPNR-----FFTASIPTHRHL	300
33 )	TPLEQFMEIKEQNRDVLLLMEVGYKYKFHGEGAKTASRELGIVAFPNR-----FFTASIPTHRHL	300

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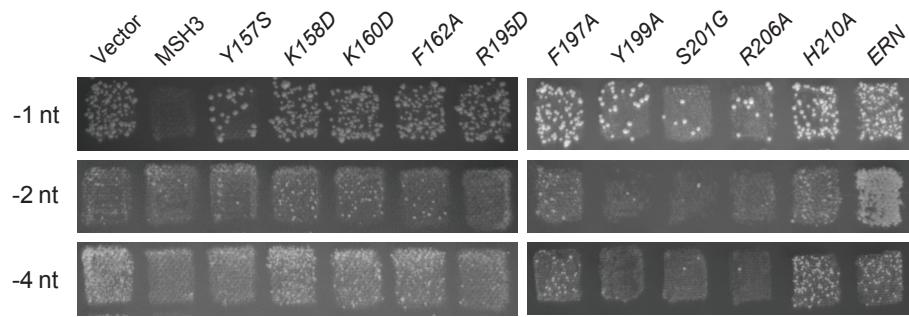
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1)	NVHLERLVHHNLKVAVVEQAETSAIKKHD-----PGASKSSVFERKISNVFTK	284
2)	HIHVKRLLIQAGHKVGVVRQIETRALKAA-----SKNAYTPFVRKLTALYTA	341
3)	FVHVRRLVAKGYKVGVVQQTETAALKAI-----GDNRSSLFSRKLTALYTK	327
4)	EYWAAQFIQMGYKVAKVDQRESMLAKEMR-----EGSKGIVKRELQCILTS	420
5)	GRYSDSLVQKGYKVARVEQTETPEMMEARCRKMAHISKYDRVVRREICRIITK	519
6)	ENYLAKLVNQGESVAICEQIGDPATS-----KGPVERKVVIRVT-	115
7)	EAYAERLLKMGFRLAVADQVEPAAA-----EGLVRREVTLQTT-	116
8)	EVHLQRLMHHNLKVGVVEQETSAVKKNSG-----TSSSVFSREVNTNIFTR	261
9)	HVHLQRLVFFNYKVAVVEQETTSALKNN-----NSGSLFTREIKNIFTK	269
10)	KVHLERLVRSGLKVAVVEQSETATKKFDNS-----KAKTSVFERKITGTYSK	271
11)	HIHLQRILSHGYKVGVVQIESTIVKQIEKT-----SKSSDVMRREVTGVYTK	220
12)	HIHLKRLLNQGLKVGVAQQTETAALKSIDST-----NKSG-LFEREITGVYTK	231
13)	DFYTKQLVEKGFKVGHVQQMEVAALKVEN-----KKGPMIRELTNTFTK	260
14)	FVHVRRLVAKGYKVGVVQQTETAALKAIGD-----NRSSLFSRKLTALYTK	222
15)	FVHVRRLVAKGYKVGVVQQTETAALKAIGD-----NRSSLFSRKLTALYTK	299
16)	FVHVRRLVAKGYKVGVVQQTETAALKAVGD-----NKSSLFSRKLTALYTK	325
17)	FVHVRRLVAKGYKVGVVQQTETAALKAIGD-----NKSSVFSRKLTALYTK	294
18)	FVHVRRLVAKGHKVGVIKQMETAALKAGE-----NKSSLFSRKLTALYTK	288
19)	DYHLERLINFGGLKVAVVVRQQTETAALKSTSS-----SRNTLFDRRVARVLTK	210
20)	HVHVKRLLVAAGHKVGVVRQLETAALKAAGD-----NRNAPFVRKLTNVYTK	315
21)	HVHVKRLLITAGHKVGIVRQIETAAALKAAGD-----NRNAPFVRKLTNVYTK	326
22)	HVHVKRLLVSAGHKVGVVRQMETAALKAAGD-----NRNAPFGRKLTNVYTK	318
23)	HVHVKRLLVSAGYKVGVVVRQLETAALKAAGD-----NRNAPFSRKLTNVYTK	330
24)	HVHVKRLLRAGHKVGVVVRQLETAALKAAGD-----NRNAPFERKLTHLYTK	390
25)	PVHAKRLVAAGYKIGIVRQQTETAALKAGD-----NRNAPFVRKLTNVYTK	330
26)	PIHAKRLVAAGHKIGIVRQQTETAALKAGD-----NRNTPFVRKLTNVYTK	327
27)	SVHVKRLLVAAGHKVGVVVRQLETAALKAGD-----NRNAPFVRKLTNIYTK	344
28)	NVHAKRLVAAGHKVGVVVRQVETAALKAGD-----NRNAPFVRKLTNVYTK	328
29)	PVHAKRLVAAGYKVGVVVRQIETAAALKAGD-----NRNAPFVRKLTNVYTK	306
30)	PVHAKRLVAAGYKVGVVVRQIETAAALKAGD-----NRNAPFVRKLTNVYTK	322
31)	PVHAKRLVAAGHKVGVVVRQIETAAALKAGD-----NRNAPFVRKLCEVYTK	346
32)	HIHVKKLLSLGYKVGVITQTETAALKKIGD-----NRNAPFARKLTHLFTA	346
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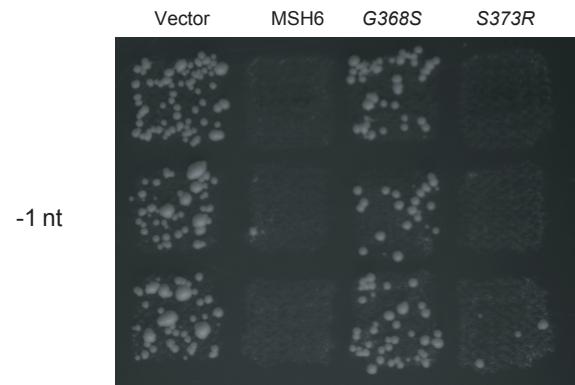
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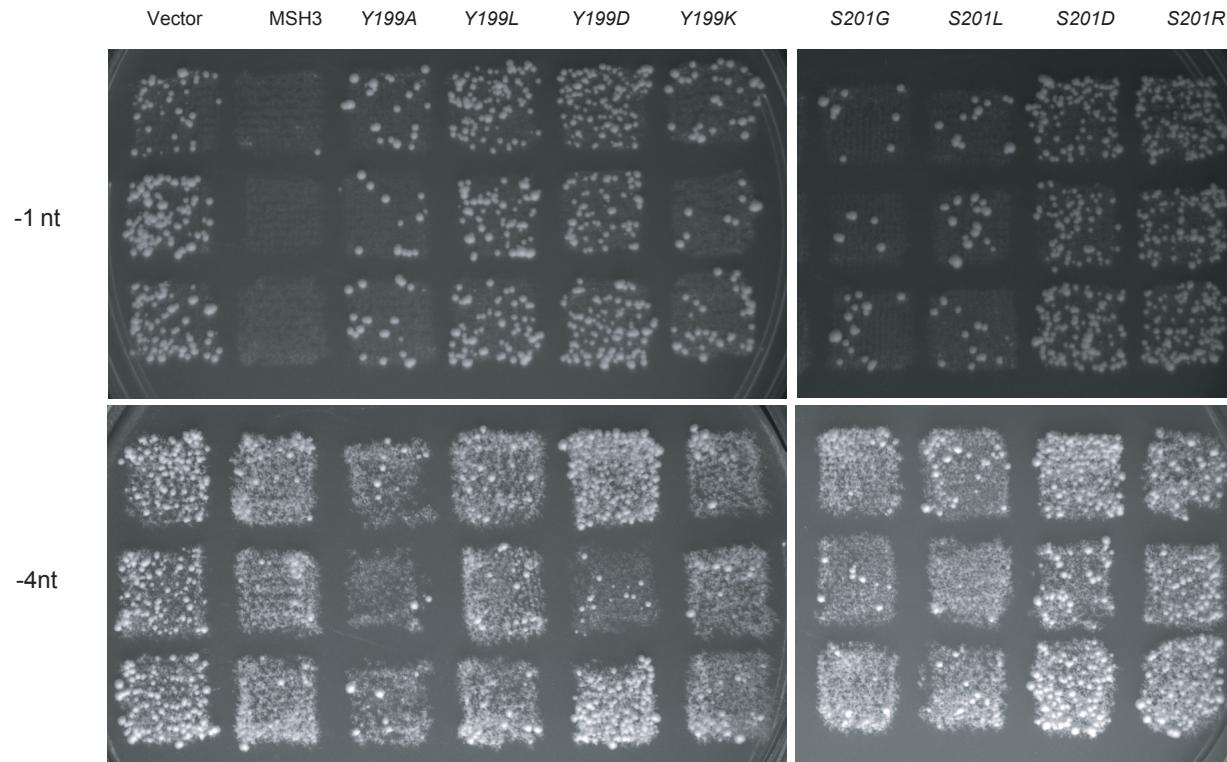
## Supplementary Figure 2.



**Supplementary Figure 3.**



**Supplementary Figure 4.**



**Supplementary Figure 5.**

